

# **CURRENT PROTOCOLS IN MOLECULAR BIOLOGY**

**VOLUME 1**

## **EDITORIAL BOARD**

**Frederick M. Ausubel**  
Massachusetts General Hospital & Harvard Medical School

**Roger Brent**  
Massachusetts General Hospital & Harvard Medical School

**Robert E. Kingston**  
Massachusetts General Hospital & Harvard Medical School

**David D. Moore**  
Massachusetts General Hospital & Harvard Medical School

**J.G. Seidman**  
Harvard Medical School

**John A. Smith**  
University of Alabama

**Kevin Struhl**  
Harvard Medical School

## **GUEST EDITORS**

**Lisa M. Albright**  
DNA Sequencing

**Donald M. Coen**  
Harvard Medical School  
Polymerase Chain Reaction

**Ajit Varki**  
University of California San Diego  
Glycoproteins

## **SERIES EDITOR**

**Virginia Benson Chanda**



John Wiley & Sons, Inc.

CORE 13 (S36)

Copyright © 1994–1997 by John Wiley & Sons, Inc.

Copyright © 1987–1994 by Current Protocols

All rights reserved. Published simultaneously in Canada.

Reproduction or translation of any part of this work beyond that permitted by Section 107 or 108 of the 1976 United States Copyright Act without the permission of the copyright owner is unlawful. Requests for permission or further information should be addressed to the Permissions Department, John Wiley & Sons, Inc.

While the authors, editors, and publisher believe that the specification and usage of reagents, equipment, and devices, as set forth in this book, are in accord with current recommendations and practice at the time of publication, they accept no legal responsibility for any errors or omissions, and make no warranty, express or implied, with respect to material contained herein. In view of ongoing research, equipment modifications, changes in governmental regulations, and the constant flow of information relating to the use of experimental reagents, equipment, and devices, the reader is urged to review and evaluate the information provided in the package insert or instructions for each chemical, piece of equipment, reagent, or device for, among other things, any changes in the instructions or indication of usage and for added warnings and precautions. This is particularly important in regard to new or infrequently employed chemicals or experimental reagents.

*Library of Congress Cataloging in Publication Data:*

Current protocols in molecular biology. 3 vols.

1. Molecular biology—Technique. 2. Molecular biology—Laboratory manuals. I. Ausubel, Frederick M.

QH506.C87 1987 574.8'8'028 87-21033

ISBN 0-471-50338-X

Printed in the United States of America

20 19 18 17 16 15 14 13

# IN SITU HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY

# 14

<b>INTRODUCTION</b>	<b>14.0.3</b>
<b>14.1 Fixation, Embedding, and Sectioning of Tissues, Embryos, and Single Cells</b>	<b>14.1.1</b>
Basic Protocol: Paraformaldehyde Fixation and Paraffin Wax Embedding of Tissues and Embryos	14.1.1
Alternate Protocol: Fixation of Suspended and Cultured Cells	14.1.3
Support Protocol: Perfusion of Adult Mice	14.1.4
Support Protocol: Sectioning Samples in Wax Blocks	14.1.4
Reagents and Solutions	14.1.6
Commentary	14.1.7
<b>14.2 Cryosectioning</b>	<b>14.2.1</b>
Basic Protocol: Specimen Preparation and Sectioning	14.2.1
Support Protocol: Fixation of Cryosections for In situ Hybridization	14.2.5
Support Protocol: Tissue Fixation and Sucrose Infusion	14.2.6
Commentary	14.2.6
<b>14.3 In situ Hybridization to Cellular RNA</b>	<b>14.3.1</b>
Basic Protocol: Hybridization Using Paraffin Sections and Cells	14.3.1
Alternate Protocol: Hybridization Using Cryosections	14.3.5
Support Protocol: Synthesis of <sup>35</sup> S-Labeled Riboprobes	14.3.7
Support Protocol: Synthesis of <sup>35</sup> S-Labeled Double-Stranded DNA Probes	14.3.8
Reagents and Solutions	14.3.8
Commentary	14.3.11
<b>14.4 Detection of Hybridized Probe</b>	<b>14.4.1</b>
Basic Protocol: Film Autoradiography	14.4.1
Basic Protocol: Emulsion Autoradiography	14.4.1
Support Protocol: Preparation of Diluted Emulsion for Autoradiography	14.4.2
Commentary	14.4.3
<b>14.5 Counterstaining and Mounting of Autoradiographed In situ Hybridization Slides</b>	<b>14.5.1</b>
Basic Protocol: Giemsa Staining	14.5.1
Alternate Protocol: Hematoxylin/Eosin Staining	14.5.2
Alternate Protocol: Toluidine Blue Staining	14.5.3
Alternate Protocol: Hoechst Staining	14.5.3
Reagents and Solutions	14.5.4
Commentary	14.5.5
<b>14.6 Immunohistochemistry</b>	<b>14.6.1</b>
Basic Protocol: Immunofluorescent Labeling of Cells Grown as Monolayers	14.6.1
Alternate Protocol: Immunofluorescent Labeling of Suspension Cells	14.6.2
Basic Protocol: Immunofluorescent Labeling of Tissue Sections	14.6.3
Alternate Protocol: Immunofluorescent Labeling of Tissue Sections Using Coplin Jars	14.6.4
Alternate Protocol: Immunofluorescent Labeling Using Streptavidin-Biotin Conjugates	14.6.5
Alternate Protocol: Immunogold Labeling of Tissue Sections	14.6.6
Alternate Protocol: Immunoperoxidase Labeling of Tissue Sections	14.6.6
Alternate Protocol: Immunofluorescent Double-Labeling of Tissue Sections	14.6.7
Reagents and Solutions	14.6.7
Commentary	14.6.8

*continued*

**14.0.1**

**SDS electrophoresis buffer, 5×**

15.1 g Tris base  
72.0 g glycine  
5.0 g SDS  
H<sub>2</sub>O to 1000 ml  
Dilute to 1× or 2× for working solution, as appropriate

*Do not adjust the pH of the stock solution, as the solution is pH 8.3 when diluted. Store at 0° to 4°C until use (up to 1 month).*

**SED (standard enzyme diluent)**

20 mM Tris-Cl, pH 7.5  
500 µg/ml bovine serum albumin (Pentax Fraction V)  
10 mM 2-mercaptoethanol  
Store up to 1 month at 4°C

**Sodium acetate, 3 M**

Dissolve 408 g sodium acetate·3H<sub>2</sub>O in 800 ml H<sub>2</sub>O  
Add H<sub>2</sub>O to 1 liter  
Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid

**Sodium acetate buffer, 0.1 M**

*Solution A:* 11.55 ml glacial acetic acid/liter (0.2 M).  
*Solution B:* 27.2 g sodium acetate (NaC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>·3H<sub>2</sub>O)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H<sub>2</sub>O to 100 ml. (See Potassium acetate buffer recipe for further details.)

**Sodium phosphate buffer, 0.1 M**

*Solution A:* 27.6 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O per liter (0.2 M).  
*Solution B:* 53.65 g Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H<sub>2</sub>O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

**SSC (sodium chloride/sodium citrate), 20×**

3 M NaCl (175 g/liter)  
0.3 M Na<sub>3</sub>citrate·2H<sub>2</sub>O (88 g/liter)  
Adjust pH to 7.0 with 1 M HCl

**STE buffer**

10 mM Tris-Cl, pH 7.5  
10 mM NaCl  
1 mM EDTA, pH 8.0

**TAE (Tris/acetate/EDTA) electrophoresis buffer**

*50× stock solution:*

242 g Tris base  
57.1 ml glacial acetic acid  
37.2 g Na<sub>2</sub>EDTA·2H<sub>2</sub>O  
H<sub>2</sub>O to 1 liter

*Working solution, pH ~8.5:*

40 mM Tris-acetate  
2 mM Na<sub>2</sub>EDTA·2H<sub>2</sub>O

**TBE (Tris/borate/EDTA) electrophoresis buffer**

*10× stock solution, 1 liter:*

108 g Tris base (890 mM)  
55 g boric acid (890 mM)  
40 ml 0.5 M EDTA, pH 8.0 (20 mM)